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Woody litter protects peat carbon stocks during drought

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WOODY LITTER PROTECTS PEAT CARBON STOCKS DURING DROUGHT

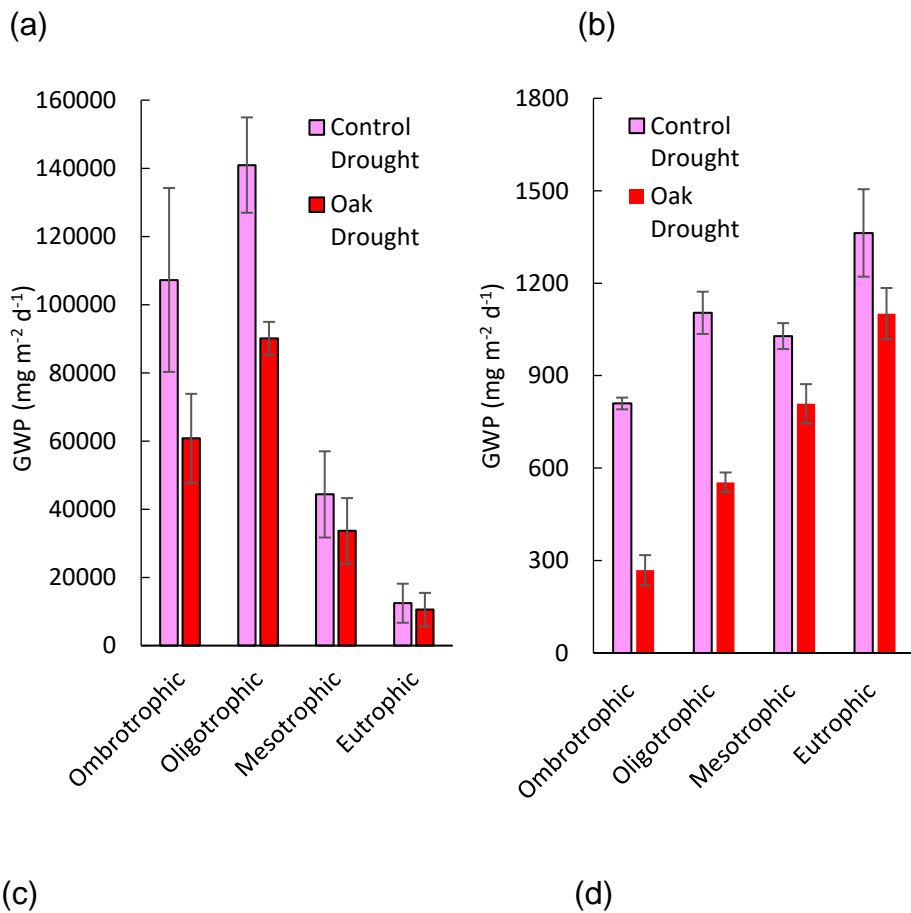
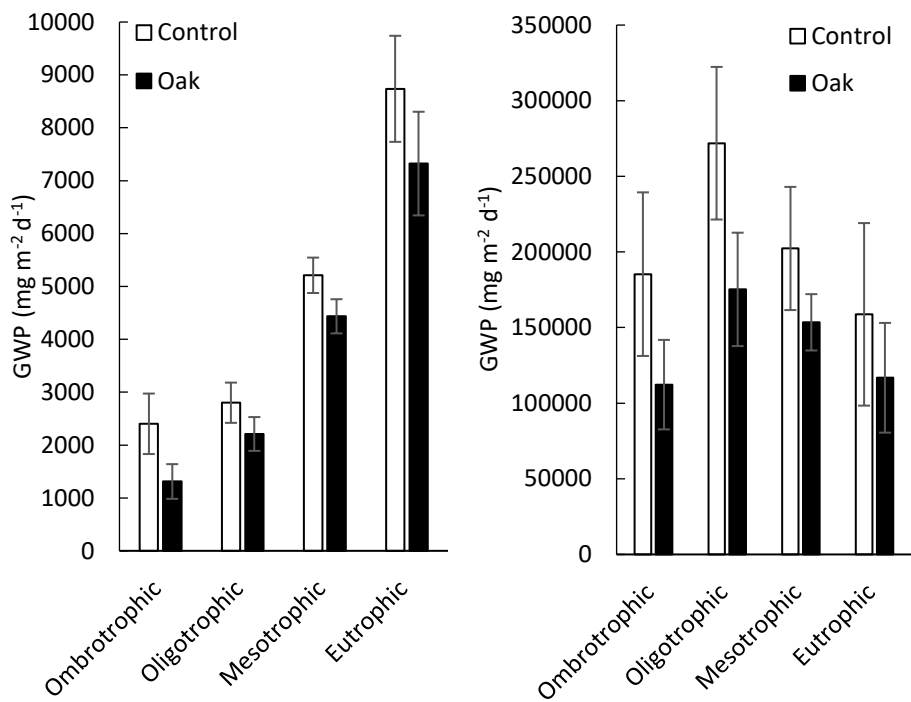
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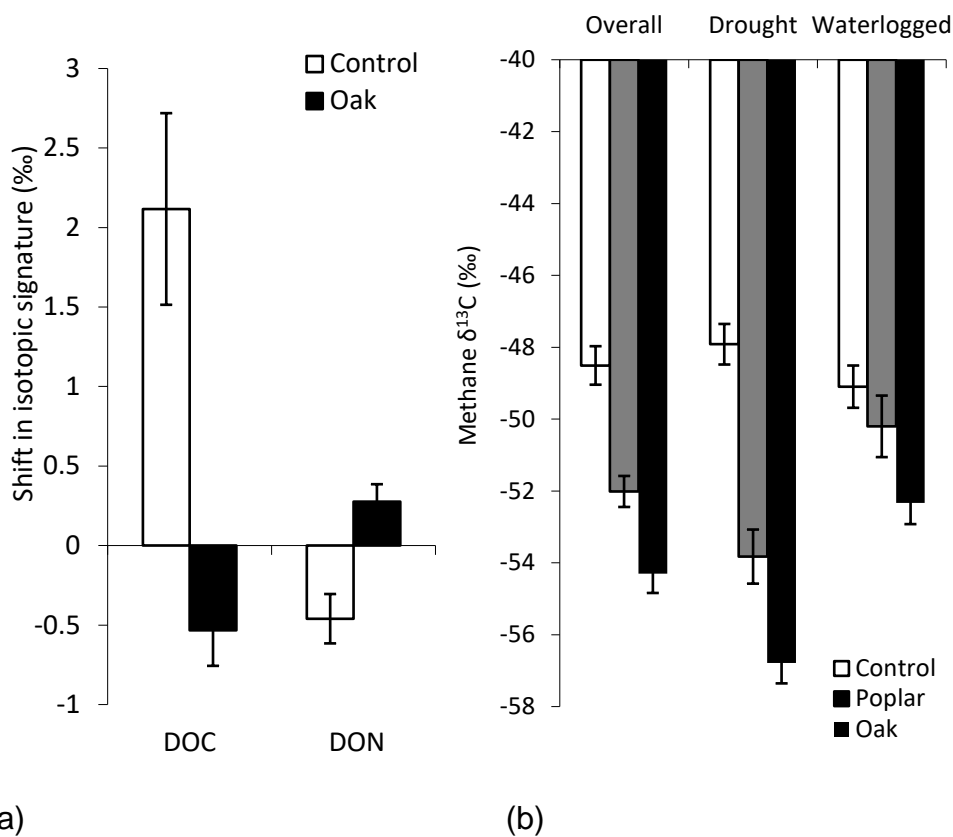
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SUPPLEMENTARY INFORMATION

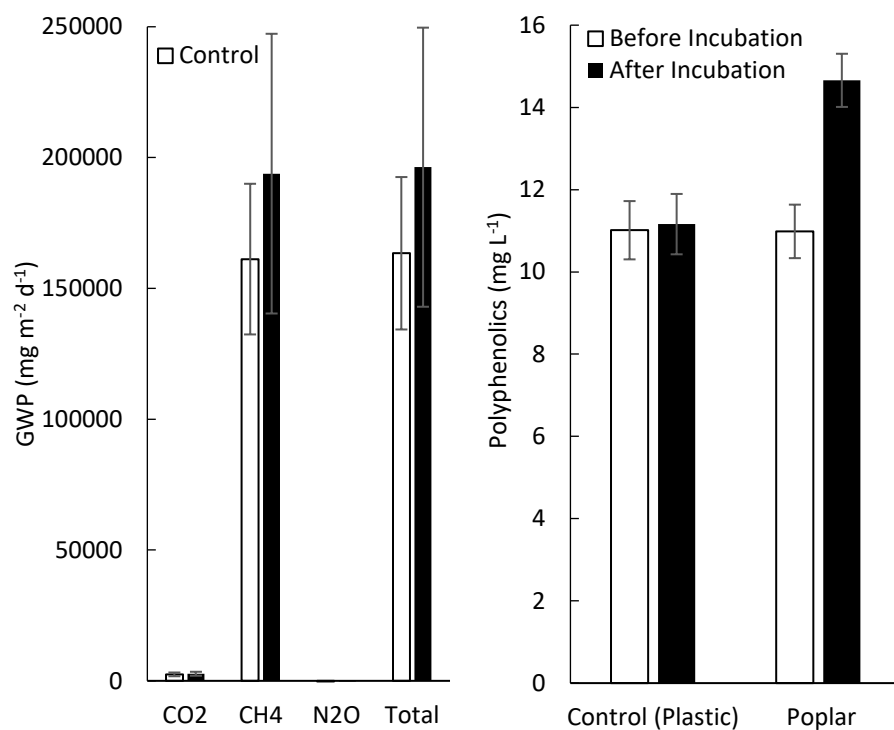
SUPPLEMENTARY FIGURE 1-4



Supplementary figure 1. Effect of Oak on greenhouse gas emissions across peat types. (a) CO₂ contributions to global warming potential (GWP CO₂eq) across a range of peatland types over 3 years, (b) CH₄, contributions to GWP, and contributions to GWP of (c) CH₄, and (d) N₂O under simulated severe drought (1/100 years, 60 days duration, water table 30cm below surface). N=6 plant-peat mesocosms per trophic level and treatment, error bars denote SEM. N₂O fluxes were especially related to trophic status and sensitive to inhibition by polyphenolics (Figure 3a). (GLM) ANOVA was used to determine whether treatment induced a significant effect at each trophic level. Overall N₂O in the control compared with Oak supplemented treatment was significantly different (Oak F=10.962, DF 43, P=0.002; Trophic level F=667.694, DF 43, P=0.000) and similarly, in the drought phase (Oak F=52.310, P=0.000; trophic level 27.551, P=0.000). Paired T-tests were significant at each trophic level, both overall and under drought conditions within trophic system and across systems (i.e., using all data with a comparison between treatment and control). Regression analysis using categorical predictor coding (DF3) shows overall control N₂O: R²=98.96%, F=632.29, P=0.000; overall Oak R²=97.63%, F=274.69, P=0.000, and, drought: control N₂O *versus* trophic status: R²=53.66%, F=7.72, P=0.001; drought Oak R²=84.00%, F=35.01, P=0.000. GLM ANOVA, with wood and trophic level as the model, showed that the interactive effect was additive rather than synergistic.



Supplementary figure 2. Effect of wood additions on indicators of decomposition. (a) Shifts in isotopic signature of leachate dissolved organic carbon ($\delta^{13}\text{C}$ -DOC) and dissolved organic nitrogen ($\delta^{15}\text{N}$ -DON), as a result of Oak addition compared with controls, and, (b) $\delta^{13}\text{C}$ -CH₄ with Oak and Poplar additions compared with the control. $\delta^{13}\text{C}$ -DOC overall 2.65‰, (T=-3.97, P=0.011) and $\delta^{15}\text{N}$ -DON overall -0.74‰ (T=3.82, P=0.012) as a result of Oak addition. $\delta^{13}\text{C}$ -CH₄ Oak: overall -5.80‰ (T=-5.16, P=0.004); drought: -8.87‰ (T=-7.88, P=0.001); waterlogged: -3.24‰ (T=-3.62, P=0.015). Poplar: overall -3.51‰ (T=5.19, P=0.003); drought: -5.91‰ (T=4.93, P=0.004); waterlogged: -1.1‰ (ns T=1.29, P=0.253). N=6 ombrotrophic microcosms, error bars denote SEM.



Supplementary figure 3. Effects of disturbance and incubation in mesocosms. a) Negligible effects of disturbance on contributions to global warming potential (GWP in CO₂eq) from each of the greenhouse gases, using high dosage (20 units) plastic pieces added to peat collected from ombrotrophic peat, compared with controls. b) Polyphenolics concentrations before and after incubation with plastic (control) showing negligible effect of the artificial hydrological regime in mesocosms and disturbance effects, or Poplar wood, showing the accumulation of phenolics.

SUPPLEMENTARY TEXT 1-4

SUPPLEMENTARY TEXT 1: EFFECTS OF PEAT TYPE ON EXOGENOUS CARBON STABILITY

N₂O uptake

The ombrotrophic site was a net sink when averaged overall, but a source during drought (Figure 3c) and this was confirmed *in-situ* for the oligotrophic site (Figure 5a). Our ombrotrophic system was relatively pristine and many ombrotrophic virgin sites show low emissions or net consumption^{1,2,3}.

N₂O production and consumption mechanisms have received attention recently, due to increasing emissions of this potent GHG and cause of stratospheric ozone depletion^{4,5}. N₂O-reducing microorganisms are the only known biological sink for N₂O and can be both denitrifying and non-denitrifying^{4,5}. Their diversity has been found to be higher than previously thought, with the latter garnering particular interest as a potential global warming mitigation strategy, due to the fact that they could truly remove N₂O from the atmosphere without contributing to formation^{4,5}. Just how timber additions promote reduced N₂O production or, greater 'scrubbing' from the atmosphere, requires further research, but microbial N₂O-reduction has been linked with energy conservation and detoxification⁴.

Isotopic fractionation

During the control incubation we observed shifts in $\delta^{13}\text{C}$ signature that are characteristic of normal decomposition processes; an enrichment in the $\delta^{13}\text{C}$ -DOC signature of 2.12‰ (Supplementary figure 2a), consistent with the formation of microbial biomass from depleted carbon sources, and preferential consumption of ^{12}C ⁽⁶⁾. Contemporaneously, $\delta^{15}\text{N}$ -DON signatures became more negative (-0.46‰ Supplementary figure 2a), indicative of depleted, microbial amino acids⁷. However, following supplementation (even with less effective Poplar: Supplementary figure 2a), these shifts were reversed, indicating an inhibition of normal DOM processing (and hence fractionation), resulting in preservation of a similar signature to that of the initial bulk peat⁷. Similarly, isotopic data from *in-vitro* work suggests

that supplementation, with Poplar and especially with Oak, favoured CO₂ reduction, rather than acetate fermentation CH₄ production pathways, particularly in the drought phase (Supplementary figure 2b), reminiscent of less reactive, older peat⁶. However, more research is needed, since there may be other drivers of the changes in signatures.

SUPPLEMENTARY TEXT 2: MECHANISMS INHIBITING DECOMPOSITION

Inhibition of extracellular enzyme activities

The only exception was phosphatase, which appeared to be modestly stimulated by the exogenous carbon additions, and while this requires more research, this could be explained by the fact that phenolic compounds require phosphorylation before crossing microbial membranes⁸.

Deprivation of substrates required for growth

The potential for reduced primary production in the long-term, as a result of supplementation requires further research, since this could negate the beneficial effects due to reduced decomposition in the most extreme ombrotrophic systems, if atmospheric nutrient deposition is limiting. However, given that N deposition and other eutrophication drivers are generally increasing globally, this 'nutrient scavenging' effect may be valuable in adding resilience.

SUPPLEMENTARY TEXT 3: PROTECTION OF HOST ECOSYSTEM SOIL CARBON DURING DROUGHT

Algae and fungi were not apparently affected by wood supplements, consistent with the consensus that the minimum inhibitory concentration (MIC) of tannins for bacteria (0.012-1 g L⁻¹) is generally lower than that of fungi depending on species (>0.5 g L⁻¹)³².

Similar effects were found using *in-vitro* leachate additions in follow-up experiments from Oak, Poplar, Alder, Sapele, Black Locust and also tannic acid (Supplementary table 3), but again, it was most pronounced using wood types with high polyphenolic contents. However, more research is needed on the character of the leached polyphenolics and their potential for microbial inhibition as a substitute for wood amendments, along with industrial waste products.

SUPPLEMENTARY TEXT 4: CARBON SEQUESTRATION AND ECOSYSTEM RESTORATION APPLICATIONS

Sphagnum peatlands cover 3.8-4.1 million km²(2,39). If we assume half this area is accessible for supplementation and extrapolate from our small-scale experiments, as an indicator of the potential for decreased GWP, then ca. 0.9-1.0Gt CO₂-eq y⁻¹ (Figure 5, Supplementary table 4, *methods) could be saved. This reduction in GWP arises solely from enhanced carbon retention in the host peat - it does not include the supplement carbon, which will depend on the tree species (below) and host ecosystem nutrient status but also hydrology. Furthermore, other peatland types were also found to store carbon efficiently (Supplementary table 4).

Oak's widespread nature, abundance and predominance as ancient archaeological remains¹⁹ make it a candidate for eco- and geo-engineering research, although fast growing, non-durable, commonly used species (such as Poplar) could be more useful for intensive carbon sequestration⁴⁶. Tropical hardwoods that possess higher levels of polyphenolics than temperate hardwoods⁹ could be particularly effective in terms of polyphenolic leaching and, therefore, host ecosystem soil carbon protection in warm and wet climates. We found Sapele to be efficient at preserving exogenous carbon with greater polyphenolic leaching than Oak (Supplementary table 4).

Disturbance due to insertion was negligible at the small-scale (Supplementary figure 3), whilst GWP was much reduced under drained systems (Figure 5c, Supplementary table 4), but this needs further investigation at high doses and the landscape-scale.

Alder would be a prime candidate for the geo-paludiculture strategy in temperate zones, because it is a natural wetland species, widespread across Europe (from Scandinavia to Morocco and Algeria), producing Alder swamps or carrs and known to be particularly durable when submerged, comparable with Oaks, Black Locust and also Larch⁵³. It is already used to produce biomass for direct combustion, pyrolysis and biomass-to-liquid fuel, but also produces high value wood used for carpentry and furniture production⁴². Furthermore, it has been found to actively form peat¹⁰ and, if held at a water regime at 10 cm below the surface, generates a commercially viable timber harvest and saves 5316kg ha⁻¹ y⁻¹ CO₂ equivalents (0.53Kg m⁻² y⁻¹)^{40,11}. Pine has been found to possess particularly potent antimicrobial properties¹² and therefore may be a candidate for further research.

SUPPLEMENTARY TABLE 1-3

	Ombrotrophic	Oligotrophic	Mesotrophic	Eutrophic
Identifier	Site 1	Site 2	Site 3	Site 4
UK NGR	SH816440	SN820866	SH497826	SH643625
Description	Upland Bog	Upland Flush Mire	Lowland calcareous Fen	Mid-altitude Riparian Wetland
Name	Migneint	Plynlimon	Gors Goch	Nant Ffrancon
Soil type	Organic	Organic	Organic	Mineral
pH	4.0	4.8	6.0	5.1
Bulk density (g cm⁻³)	0.05	0.06	0.08	0.16
Organic Matter Content	99%	95%	94%	90%
Phenolics (mg L⁻¹)	1.84 (0.13)	1.71 (0.15)	0.14 (0.04)	0.48 (0.19)
DOC (mg L⁻¹)	83.64(7.16)	79.9(6.22)	19.91 (1.52)	37.28 (9.73)
Nitrate (mg L⁻¹)	0.04 (0.02)	0.05 (0.01)	0.08(0.01)	0.15 (0.03)
Phosphate (mg L⁻¹)	8.64 (1.25)	0.03 (0.05)	0.05 (0.03)	0.03 (0.02)
Sulphate (mg L⁻¹)	3.38 (0.65)	1.28 (0.23)	0.85 (0.38)	2.23 (0.63)
Calcium (mg L⁻¹)	1.25 (0.19)	2.55 (0.89)	50.05 (2.10)	4.97 (1.14)

Supplementary table 1. Peatland field site characteristics. pH, organic matter content and bulk density refer to long-term averages (>5 years, sampled monthly, in-situ). All other determinands are based on mesocosm data (over 3 years, n=6, sampled monthly), SEM in brackets.

DETERMINAND	PHASE	CONTROL	SEM	WOOD	SEM	DIFFERENCE (%)	T- VALUE	P-VALUE
Polyphenolics (mg L ⁻¹)	Overall	14.80	0.23	18.58	0.23	+25.6	-10.49	0.000
	Drought	8.46	0.88	13.46	0.79	+59.1	6.47	0.001
Phenol Oxidase (nMol g ⁻¹ min ⁻¹)	Overall	82.30	2.96	55.00	3.04	-33.2	13.11	0.000
	Drought	84.65	5.23	35.79	3.80	- 57.7	7.21	0.001
β-glucosidase (μMol g ⁻¹ min ⁻¹)	Overall	30.76	0.28	(23.52)	0.81	- 23.5	8.78 (98.54)	0.000 (0.000)
	Drought	29.32	1.07	20.03	1.03	- 31.7	8.06	0.000
N-acetyl-β-D-glucosaminidase (μMol g ⁻¹ min ⁻¹)	Overall	11.85	0.84	6.33	0.70	-46.6	3.74	0.013
	Drought	10.56	0.72	5.05	0.59	-52.2	4.41	0.007
Phosphatase (μMol g ⁻¹ min ⁻¹)	Overall	49.04	1.16	52.23	1.01	+6.5	-2.20	0.079
	Drought	50.75	1.16	54.24	1.43	+6.9	-2.69	0.043
Bacterial Growth (DPM)	Overall	30721.3	3048.8	16891.7	941.9	-45.0	5.05	0.004
	Drought	26243.5	2760.5	15567.1	2338.2	-40.7	2.99	0.030

Catechol 2,3-dioxygenase ($\mu\text{Mol min}^{-1} \text{mg}^{-1}\text{protein}$)	Overall	1652.80	70.68	989.39	72.68	- 40.1	6.30	0.003
	Drought	1497.39	160.88	770.65	67.24	-48.5	3.38	0.028
Bacterial Abundance (Cells x 10^6)	Overall	0.66	0.01	0.53	0.02	-19.7	5.81	0.002
DOC (mg L^{-1})	Overall	73.52	1.44	55.89	1.66	-24.0	39.99	0.000
	Drought	55.77	2.50	39.93	2.58	-28.4	7.97	0.001
DON (mg L^{-1})	Overall	2.41	0.28	1.56	0.23	-35.5	2.90	0.044
	Drought	1.49	0.28	0.61	0.23	-59.0	4.74	0.009
NH₄ (mg L^{-1})	Overall	(2.85)	0.33	2.36	0.34	-17.2	3.65 (-11.70)	0.022 (0.000)
	Drought	1.09	0.38	0.77	0.15	-29.3	1.26	0.277
Iron (mg L^{-1})	Overall	0.276	0.02	0.198	0.019	-28.3	6.29	0.003
	Drought	0.218	0.016	0.156	0.014	-28.4	6.39	0.003

Supplementary table 2. Effects of Oak addition on microbial inhibitors, enzyme activities and substrates, overall (3 years) and during drought (simulated 1/100 years drought 60 days duration, water table 30cm below surface). Bacterial growth rates indicated by ^3H Thymidine incorporation (Disintegrations Per Minute). N=6 peat microcosms per treatment and significance was determined using the Paired T-test.

Brackets denote Johnson Transformed data where normality could not be assumed. Red font shows where the difference between control and treatment is larger in the drought than the waterlogged phase.

Supplement	Polyphenolics (mg L ⁻¹)	% GWP reduction	P-value
Oak (<i>Quercus robur</i>)	13.3 (0.5)	198	0.005
Poplar (<i>Liriodendron tulipifera</i>)	9.5 (0.9)	89	0.049
Alder (<i>Alnus glutinosa</i>)	11.9 (0.4)	171	0.002
Black Locust (<i>Robinia pseudoacacia</i>)	14.2 (0.7)	163	0.04
Sapele (<i>Entandrophragma cylindricum</i>)	16.1 (0.6)	199	0.01
Tannic acid	10	90	0.05

Supplementary table 3. Concentrations of leachate polyphenolics from various wood types incubated in ultra-pure water for 12 months, and their effect on peatland global warming potential (GWP CO₂eq) compared with tannic acid. N=5 ombrotrophic *in-vitro* peat microcosms, SEM is shown in brackets.

						Sphagnum Peatland area			
						Lower	Upper	Lower	Upper
Peatland type	Approach	Difference in CO₂eq (mg m⁻² d⁻¹)	g m⁻² d⁻¹	Kg km⁻² y⁻¹	Tg km⁻² y⁻¹	Tg y⁻¹	Tg y⁻¹	Gt y⁻¹	Gt y⁻¹
Ombrotrophic	Mesocosm	74337	74	27133042	0.03	103106	111245	103	111
		49996	50	18248575	0.02	69345	74819	69	75
Oligotrophic	Mesocosm	97248	97	35495594	0.04	134883	145532	135	146
		52924	53	19317327	0.02	73406	79201	73	79
Mesotrophic	Mesocosm	49696	50	18139142	0.02	68929	74370	69	74
		11554	12	4217159	0.00	16025	17290	16	17
Eutrophic	Mesocosm	43598	44	15913157	0.02	N/A	N/A	N/A	N/A
		2778	3	1013918	0.00	N/A	N/A	N/A	N/A
Fallen oak	Mesocosm	14689	15	5361464	0.01	20374	21982	20	22
		9237	9	3371419	0.00	12811	13823	13	14
Pristine oligotrophic*	<i>In-situ</i>	1273	1	464682	0.00	1766	1905	2	2
		2362	2	862130	0.00	3276	3535	3	4
Pristine v Drained**	<i>In-situ</i>	431544	432	157513696	0.16	598552	645806	599	646

Supplementary table 4. Summary of effects of geoengineering and ecoengineering (**bold**) on peatland global warming potential (CO₂eq). Shading denotes severe drought phase (natural or simulated 1/100 years drought 60 days duration, water table 30cm below surface). *Figure used in discussion since it is most likely to be representative of *in-situ* effect of wood addition.**Effect of natural vegetation changes as a result of drainage. Figures for eutrophic peat were not extrapolated using the area of *Sphagnum* peatlands because these tend to be mainly ombrotrophic, oligotrophic or mesotrophic. 1 Gt = 1x10¹⁵ g or 1Pg.

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